We claim:

1. An aqueous composition buffered to a pH of from about 7 to about 9, which comprises:

(a) first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of human cytomegaloviral DNA (hCMV DNA) and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

(b) third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA, the third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along the opposing strands by from 90 to 400 nucleotides,

each of said first, second, third and fourth primers having a T_m within the range of from about 65 to about 74°C, all of said primer T_m's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides.

2. The composition of claim 1 wherein each of said primers is present at a concentration of at least about 0.075 µmolar, and said composition further comprises

a thermostable DNA polymerase present at from about 0.1 to about 50 units/100 µl,

a DNA polymerase cofactor present at from about 2 to about 15 mmolar, and

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a dNTP present at from about 0.25 to about 3.5 mmolar.

3. The composition of claim 1 wherein each of said first, second, third and fourth primers has a T_m within the range of from about 67 to about 74°C, all of said primer T_m's being within about 2°C of each other.

4. The composition of claim 1 wherein said first and second primers have nucleotide lengths in the range of from 20 to 35, which lengths differ from each other by no more than 2 nucleotides.

5. The composition of claim 1 wherein said first and second primers have the same length in the range of from 20 to 30 nucleotides.

6. The composition of claim 1 wherein said T_m values are calculated using the formula:

$$T_m\ (°C) = 67.5 + 0.34(\%\ G + C) - 395/N$$

wherein G and C represent the number of guanine and cytosine nucleotides, respectively, and N represents the total number of nucleotides.

7. The composition of claim 6 wherein said third and fourth primers are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a DNA different from hCMV DNA and which is associated with an infectious agent.

8. The composition of claim 7 wherein said third and fourth primers are specific to and hybridizable with said third and fourth nucleic acid sequences which are in opposing strands of a DNA selected from the group consisting of a retroviral DNA, *Mycobacterium tuberculosis* DNA, *Mycobacterium avium* DNA, Epstein Barr viral DNA, respiratory syncytial viral DNA, *Pneumocystis carinii* DNA and hepatitis DNA.

9. The composition of claim 1 wherein said first (a) and second (b) primers are selected from the group of primer sets consisting of:

Primer set 1:

(a) 5'-GAGGCTATTG TAGCCTACAC TTTGG-3'

(b) 5'-CAGCACCATC CTCCTCTTCC TCTGG-3',

and

Primer set 2:

(a) 5'-TGCACTGCCA GGTGCTTCGG CTCAT-3'

(b) 5'-CACCACGCAG CGGCCCTTGA TGTTT-3'.

10. The composition of claim 1 wherein one or both of said first and second primers, and one or both of said third and fourth primers, are labeled with the same or different specific binding moiety.

11. The composition of claim 11 wherein said labeled primers are labeled with biotin.

12. A diagnostic test kit for the amplification of human cytomegaloviral DNA and a second target DNA comprising, in separate packaging:

a) an aqueous composition buffered to a pH of from about 7 to about 9, which comprises:

first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of human cytomegaloviral DNA (hCMV DNA) and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA, the third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from

each other along the opposing strands by from 90 to 400 nucleotides,

each of said first, second, third and fourth primers having a T_m within the range of from about 65 to about 74°C, all of said primer T_m's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and

b) at least one additional PCR reagent.

13. The test kit of claim 12 wherein said additional PCR reagent is a thermostable DNA polymerase, a DNA polymerase cofactor or a dNTP.

14. The test kit of claim 12 further comprising

a capture reagent comprising a water-insoluble support to which is covalently attached a capture probe which is specific to a nucleic acid sequence of a strand of hCMV DNA, said capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said hCMV DNA strand at a temperature in the range of from about 40 to about 55°C, and

a second capture reagent comprising a water-insoluble support to which is covalently attached a second capture probe which is specific to a nucleic acid sequence of a strand of said second target DNA, said second capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and being hybridizable with said nucleic acid sequence of said second target DNA strand at a temperature in the range of from about 40 to about 55°C.

15. The test kit of claim 14 wherein said first capture probe is selected from the group consisting of:

5'-GGTGTCACCC CCAGAGTCCC CTGTACCCGC-3',
5'-GACACAGTGT CCTCCCGCTC CTCCTGAGCA-3',
5'-GTGGAAGGCG GCTCGCTGGA AGCCGGTCGT-3', and
5'-GAACCGAGGG CCGGCTCACC TCTATGTTGG-3'.

16. The test kit of claim 12 wherein each of said primers is present at a concentration of at least about 0.075 μmolar, and said composition further comprises

a thermostable DNA polymerase present at from about 0.1 to about 50 units/100 μl,

a DNA polymerase cofactor present at from about 2 to about 15 mmolar, and

a dNTP present at from about 0.25 to about 3.5 mmolar.

17. The test kit of claim 12 wherein one or both of said first and second primers, and one or both of said third and fourth primers are labeled with biotin, and said test kit further includes a conjugate of avidin with an enzyme and a substrate reagent which provides a detectable signal in the presence of said enzyme.

18. The test kit of claim 17 wherein said conjugate comprises avidin and peroxidase, and said substrate reagent provides a detectable colorimetric or chemiluminescent signal in the presence of peroxidase and an oxidant.

19. A diagnostic test kit for the amplification of human cytomegaloviral DNA and a second target DNA comprising, in separate packaging:

a) an aqueous composition buffered to a pH of from about 7 to about 9, and comprising first and second primers which are specific to and hybridizable

with, respectively, first and second nucleic acid sequences which are in opposing strands of hCMV DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides,

each of said first and second primers having a T_m within the range of from about 65 to about 74°C, said primer T_m's being within about 5°C of each other, and said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides,

b) third and fourth primers which are specific to and hybridizable with, respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA and which are separated from each other along said opposing strands of said second target DNA by from 90 to 400 nucleotides,

each of said third and fourth primers having a T_m within the range of from about 65 to about 74°C, said third and fourth primer T_m's being within about 5°C of each other and within about 5°C of the T_m's of said first and second primers, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and

c) at least one additional PCR reagent.

20. The test kit of claim 19 wherein said third and fourth primers are specific to and hybridizable with said third and fourth nucleic acid sequences which are in opposing strands of a DNA selected from the group consisting of a retroviral DNA, *Mycobacterium tuberculosis* DNA, *Mycobacterium avium* DNA, Epstein Barr viral DNA, respiratory syncytial viral DNA, *Pneumocystis carinii* DNA and hepatitis DNA.

21. The test kit of claim 19 further comprising:

a first capture reagent comprising a water-insoluble support to which is covalently attached a first capture probe which is specific to a nucleic acid sequence of a strand of hCMV DNA, said first capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said hCMV DNA strand at a temperature in the range of from about 40 to about 55°C, and

a second capture reagent comprising a water-insoluble support to which is covalently attached a second capture probe which is specific to a nucleic acid sequence of a strand of said second target DNA, said second capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said second target DNA strand at a temperature in the range of from about 40 to about 55°C,

said first and second capture probes having T_m's which differ by no more than about 15°C.

22. A method for the amplification and detection of human cytomegaloviral DNA and a second target DNA comprising:

A) simultaneously subjecting the denatured opposing strands of hCMV DNA and the denatured opposing strands of a second target DNA to polymerase chain reaction in the presence of:

i) an aqueous composition buffered to a pH of from about 7 to about 9, and comprising

first and second primers which are specific to and hybridizable with, respectively, first and second nucleic acid sequences which are in opposing strands of hCMV DNA and which are separated from each other along said opposing strands by from 90 to 400 nucleotides, third and fourth primers which are specific to and hybridizable with,

respectively, third and fourth nucleic acid sequences which are in opposing strands of a second target DNA which is the same as or different from hCMV DNA, the third and fourth nucleic acid sequences being different from said first and second nucleic acid sequences and being separated from each other along the opposing strands by from 90 to 400 nucleotides,

each of said first, second, third and fourth primers having a T_m within the range of from about 65 to about 74°C, all of said primer T_m's being within about 5°C of each other, said first and second primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and said third and fourth primers having nucleotide lengths which differ from each other by no more than 5 nucleotides, and

ii) the additional PCR reagents: a thermostable DNA polymerase, a DNA polymerase cofactor and at least one dNTP, any or all of said additional PCR reagents being in the same or a different composition as defined in i),

to simultaneously amplify said opposing hCMV DNA strands and the opposing second target DNA strands, and

B) simultaneously detecting at least one of said amplified hCMV DNA strands and at least one of the amplified second target DNA strands as a simultaneous determination of the presence of hCMV DNA and the second target DNA.

23. The method of claim 22 wherein each of said first, second, third and fourth primers has a T_m within the range of from about 67 to about 74°C, said primer T_m's being within about 2°C of each other.

24. The method of claim 22 wherein one or both of said first and second primers, and one or both of said third and fourth primers, are labeled with a specific binding moiety.

25. The method of claim 24 wherein said labeled primers are labeled with biotin, and detection of the resulting biotinylated amplified DNA strands for either target DNA is achieved by reacting said biotinylated amplified hCMV DNA strand with an avidin-enzyme conjugate, followed by reaction of said enzyme with a substrate reagent to produce a detectable colorimetric or chemiluminescent signal.

26. The method of claim 25 wherein said biotinylated amplified DNA strands are detected by contacting them with an avidin-peroxidase conjugate, followed by reaction of peroxidase, in the presence of an oxidant, with either: luminol to produce a detectable chemiluminescent signal, or a leuco dye to produce a detectable colorimetric signal.

27. The method of claim 23 wherein PCR is carried out for from 20 to 50 cycles.

28. The method of claim 27 wherein, in each PCR cycle, priming and primer extension are carried out at the same temperature within the range of from about 62 to about 75°C.

29. The method of claim 23 wherein one of said amplified hCMV DNA strands is captured with a capture reagent comprising a water-insoluble support to which is covalently attached a capture probe which is specific to a nucleic acid sequence of said hCMV DNA strand, said capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said hCMV DNA strand at a temperature in the range of from about 40 to about 55°C, and

one of said amplified second target DNA strands is captured with a second capture reagent comprising a second capture probe specific to a nucleic acid sequence of said second target DNA strand, said

second capture probe having from 10 to 40 nucleotides and a T_m greater than about 50°C, and is hybridizable with said nucleic acid sequence of said second target DNA strand at a temperature in the range of from about 40 to about 55°C,

said first and second capture probes having T_m's which differ by no more than about 15°C.

30. The method of claim 29 wherein said water-insoluble support for each capture reagent is a polymeric or magnetic particle having a diameter in the range of from about 0.001 to 10 μmeters, and each of said capture probes has a T_m greater than about 55°C.

31. The method of claim 29 wherein said first and second capture reagents are disposed in distinct regions on a water-insoluble substrate of a test device.

32. The method of claim 29 wherein said first capture probe is selected from the group consisting of:

5'-GGTGTCACCC CCAGAGTCCC CTGTACCCGC-3',

5'-GACACAGTGT CCTCCCGCTC CTCCTGAGCA-3',

5'-GTGGAAGGCG GCTCGCTGGA AGCCGGTCGT-3', and

5'-GAACCGAGGG CCGGCTCACC TCTATGTTGG-3'.

33. The method of claim 23 wherein said second target DNA is selected from the group consisting of a retroviral DNA, *Mycobacterium tuberculosis* DNA, *Mycobacterium avium* DNA, Epstein Barr viral DNA, respiratory syncytial viral DNA, *Pneumocystis carinii* DNA and hepatitis DNA.

34. The method of claim 23 wherein said first (a) and second (b) primers are selected from the group of primer sets consisting of:

Primer set 1:

(a) 5'-GAGGCTATTG TAGCCTACAC TTTGG-3'

(b) 5'-CAGCACCATC CTCCTCTTCC TCTGG-3',

and

Primer set 2:

(a) 5'-TGCACTGCCA GGTGCTTCGG CTCAT-3'

(b) 5'-CACCACGCAG CGGCCCTTGA TGTTT-3', and

said first capture probe is selected from the group consisting of:

5'-GGTGTCACCC CCAGAGTCCC CTGTACCCGC-3',

5'-GACACAGTGT CCTCCCGCTC CTCCTGAGCA-3',

5'-GTGGAAGGCG GCTCGCTGGA AGCCGGTCGT-3', and

5'-GAACCGAGGG CCGGCTCACC TCTATGTTGG-3'.

35. The method of claim 23 wherein each of said primers is present at a concentration of at least about 0.075 μmolar,

a thermostable DNA polymerase is present at from about 0.1 to about 50 units/100 μl,

a DNA polymerase cofactor is present at from about 2 to about 15 mmolar, and

a dNTP is present at from about 0.25 to about 3.5 mmolar.

36. The method of claim 23 wherein a third target DNA is amplified and detected simultaneously with the hCMV DNA and said second target DNA,

said third target DNA being amplified using a third set of primers wherein each primer has a T_m within the range of from about 65 to about 74°C, the primer T_m's being within 5°C of each other and within 5°C of said first, second, third and fourth primers, and the lengths of primers in said third primer set differing by no more than 5 nucleotides.

37. A diagnostic element comprising a water-insoluble, heat or ultrasonic sealable support, having disposed thereon in distinct regions thereof, a plurality of capture reagents,

each capture reagent having a capture probe specific for and hybridizable with a distinct target

DNA at a temperature of from about 40 to about 55°C, each of said capture probes having from 10 to 40 nucleotides and a T_m greater than about 50°C, and the T_m's of all capture probes differing by no more than about 15°C,

at least one of said capture probes being specific for and hybridizable with hCMV DNA.

38. An oligonucleotide defined as:

TGCACTGCCA GGTGCTTCGG CTCAT,

CATTCCCACT GACTTTCTGA CGCACGT,

TGAGGTCGTG GAACTTGATG GCGT,

GGTCATCGCC GTAGTAGATG CGTAAGGCCT,

GGAATGACGC AAGGACATAT GGGCGT,

CCCAGGTGCA CACCAATGTG GTGGAT,

GGACTGTGCG CGTTGTATAC CCTGC,

ACTCCCGAAG CGAATGGCAC GTGGA,

CATAGCTTGT GCCCGTGTGG CACGT,

CCAAGACGAG ACCGTCAGAG CTGGT,

AAGCTGTTGC CGCCATCAAA TAAACG, or

CTGCGTTAGA CCGAGAACTG TGGATAAAGG.